METHOD FOR ASSAYING CLUSTERED DNA DAMAGES

Abstract of the Disclosure

Disclosed is a method for detecting and quantifying clustered damages in DNA. In this method, a first aliquot of the DNA to be tested for clustered damages with one or more lesion-specific cleaving reagents under conditions appropriate for cleavage of the DNA to produce single-strand nicks in the DNA at sites of damage lesions. The number average molecular length (Ln) of double stranded DNA is then quantitatively determined for the treated DNA. The number average molecular length (Ln) of double stranded DNA is also quantitatively determined for a second, untreated aliquot of the DNA. The frequency of clustered damages (Φ_c) in the DNA is then calculated using the equation: $\Phi_C = 1/L_n(+enzyme) - 1/L_n(-enzyme)$ wherein $L_n(+enzyme)$ is the number average molecular length of double stranded DNA determined for the lesion-specific cleaving reagent-treated DNA, and $L_n(-enzyme)$ is the number average molecular length of double stranded DNA determined for the untreated DNA. One or more lesion-specific enzymes can be used in the method. Examples of enzymes suitable for use in the method are provided. This method is useful for detecting and quantifying clustered damages in DNA of a biological organism induced by exposure of the biological organism to a DNA-damaging agent. This is achieved by assaying a sample for clustered damages before and after exposure to a DNA-damaging agent, with the difference of the two values being a value representative of the clustered DNA damage induced by exposure of the biological organism to the DNA-damaging agent. This method can be used to detect and

quantitate DNA damaging induced by DNA-damaging agents such as X-rays, γ-rays, radon, and other known or suspected carcinogens. This method can also be used to detect and quantitate an accumulation of clustered damages in DNA of a biological organism over a period of time.

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